The Great Lakes Entomologist

Volume 7
Number 4 -- Winter 1974 *Number 4 -- Winter 1974*

Article 4

December 1974

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DIURNAL SAMPLING OF THE INSECT COMPLEX OF ALFALFA

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The appearance of the alfalfa weevil, *Hypera postica* (Gyllenhal), in Michigan in 1966 (Dowdy 1966) necessitated intensive testing of methods for its control. There is a complex of insect pests in alfalfa, and the effects of control measures for alfalfa weevil on these other pests needed to be considered. An efficient sampling method that measures as many species of the complex as possible was needed. Preliminary tests of the author showed that 20 sweeps with an insect net of 15 in. diam., a standard sampling tool for alfalfa, was satisfactory for sampling the insect complex. A means of separating the insects from the plant debris collected in the net, and information on the time of day that samples should be made were needed. Satisfactory answers to these two problems were found and are summarized here.

SEPARATING THE INSECTS

Efficient methods of sampling, labeling the samples, and separating the insects from the debris were found by a series of trial-and-errors during the 1968 and 1969 seasons. Quart-sized wide-mouthed jars 1/4 full of 70% ethanol were used. A strip of masking tape with a sample identification written on it was fastened to the top of each jar. The insect net was used to make the samples, the entire contents of the net were emptied into the jars, and the jars taken to a laboratory. The samples could be stored, with more 70% ethanol added to them as needed, until it was convenient to separate the insects from the debris.

A 10 in. diam. funnel with a 14 in. length of 5/8 in. diam. Tygon tubing fastened to it was filled with 70% ethanol. Hose clamps were placed at the upper and lower end of the tubing. A 4-mesh hardware cloth basket was placed in the funnel, the insect sample emptied into the basket, and the upper clamp opened. The basket was shaken in the ethanol to dislodge the insects from the debris. The insects sank through the ethanol into the tubing while most of the debris either floated or was trapped in the basket. Use of 70% ethanol was important as some insects floated in lower concentrations and some debris sank in higher concentrations of ethanol. The upper clamp was closed when the insects had all settled in the tubing. A 4-ounce wide-mouthed jar was placed under the tubing, the lower clamp opened, and the insects collected in the jar. The masking tape label from the quart jar was transferred to the 4-ounce jar to identify the sample. The sample could be stored until convenient to sort and count the different insect species.

A 7 in. by 11-1/2 in. enamel pan about 2 in. deep was filled with sufficient 70% ethanol to cover the insects in the sample for counting. The insect sample from the 4-ounce jar was emptied into the pan, any debris removed, the insects sorted by species and stages, and counted. Sorting was facilitated by having only enough ethanol in the pan to cover the specimens. The insects could be returned to the 70% ethanol in the 4-ounce jar for later examination when desired.

The sampling method described has proved efficient for studies of the insect complex of alfalfa and can be adapted to other studies. Its special advantages when large number of samples are taken are: speeding up the collection of samples in the field; minimizing

[1]The author expresses his gratitude to Messrs. J. Velarde and S. L. Taylor of Michigan State University for their capable assistance, and to Dr. C. Van den Brink of the United States Department of Commerce for supplying the weather data for this work. Michigan Agricultural Experiment Station Journal Article Number 6640.

escapes of insects from the net; facilitating separation of the insects from the debris; and preserving specimens for identification until time permits. The last point is very important, even when smaller numbers of samples are taken, as the identification of the specimens can be varified if there is question of their identity. This point permitted the use of inexperienced personnel, with supervision, in making the counts.

DIURNAL SAMPLING

Two large fields of vigorous alfalfa near East Lansing, Michigan, were sampled at 6:00, 10:00, 14:00, 18:00, and 21:00 hours EST on 13 August and again on 24 September, 1969. Samples of 20 sweeps with a 15 in diam. insect net were made in five areas of each field (in each corner and the central area) as suggested by Blickenstaff (1966). The samples were separated and counted as described above. Statistical analyses were made using the Chi^2 tests of independence.

There were light winds and no precipitation that would interfere with sampling during both dates. The weather during the two days was otherwise very different with 13 August being warm and dry and 24 September being cool and moist (Table 1). The collections made at 6:00 hours were at dawn or low sun, and those at 21:00 hours were made in darkness. The samples made at 18:00 hours were in full sun on 13 August and in low sun on 24 September.

The insects that were found in sufficient numbers to yield reliable results on the effect of time of collection on sampling were: grubs and adults of the alfalfa weevil; all stages of the pea aphid, *Acyrthosiphon pisum* (Harris), and tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois); and adult meadow spittlebug, *Philaenus spumarius* (L.). The potato leaf hopper, *Empoasca fabae* (Harris), that is usually abundant in mid-to-late summer was scarce in the samples. The pea aphid was too abundant in the collections made on 24 September to justify counting, and the volume, in cc, of pea aphids collected on this date was recorded. The other species and the pea aphid collected on 13 August were counted.

Except for the adult meadow spittlebugs collected on 13 August, the differences between the numbers of insects collected in the different areas of the fields were significant on both dates of sampling (Table 2). Except for the alfalfa weevil grubs on both dates, the differences between the numbers of insects collected at different times of day were significant on both dates of collection (Tables 2 and 3). The interactions of area by time were not significant on either date of collection except for the pea aphid and tarnished plant bug on 13 August (Table 2). There were significant differences in the numbers of insects collected in the different fields on the same date and in the same field on different dates with the exception that the number of alfalfa weevil grubs collected on the different dates were not significant (Table 2). The interaction of date by field was significant for all insects (Table 2).

Table 1. Summary of weather in East Lansing, Michigan, on 13 August and 24 September, 1969.

TIME	13 AUGUST TEMP.,F°	RH,%	24 SEPTEMBER TEMP.,F°	RH,%
Sunrise	5:47 hrs.		6:32 hrs.	
Sunset	19:47 hrs.		18:37 hrs.	
6:00 hrs.	63	92	50	100
10:00 hrs.	62	100	54	100
14:00 hrs.	79	45	54	100
18:00 hrs.	84	45	51	100
21:00 hrs.	80	52	48	100

Table 2. Significance of differences between samples of the alfalfa insect complex drawn in different areas, in different fields, on different days, and at different times of day*.

Factors	Date	Alfalfa weevil Grubs	Alfalfa weevil Adults	Pea Aphid	Meadow spittle-Bug	Tarnish plant Bug
Areas	13 Aug.	**	**	**	ns	**
	24 Sept.	*	**	**	*	**
Time	13 Aug.	ns	**	**	**	**
	24 Sept.	ns	**	**	**	**
Area X Time	13 Aug.	ns	ns	**	ns	*
	24 Sept.	ns	ns	ns	ns	ns
Fields	13 Aug.	**	**	**	**	**
	24 Sept.	**	**	**	**	**
Date	--	ns	**	†	**	**
Field X Date	--	*	**	†	*	**

* A * indicates significance at 5% level, a ** indicates significance at the 1% level, and "ns" indicates no statistical significance as determined by a Chi^2 test of independence.
† No analysis made as the counting methods differed in the two dates. The pea aphids were obviously more abundant on 24 September than on 13 August.

Table 3. Mean numbers of alfalfa insects collected at different times of day on 13 August and 24 September, 1969.

Insect	Date	Time, hrs. EST; 6:00	10:00	14:00	18:00	21:00
Alfalfa weevil grubs	13 Aug.	20	33	24	28	30
	24 Sept.	18	18	26	17	22
Alfalfa weevil adults	13 Aug.	70	49	34	76	160
	24 Sept.	4	17	20	47	31
Pea aphid	13 Aug.	776	1822	1430	1832	698
	24 Sept.[a]	292	362	370	322	249
Tarnished plant bug	13 Aug.	457	368	244	401	460
	24 Sept.	274	176	191	259	192
Meadow spittlebug adults	13 Aug.	17	14	7	5	16
	24 Sept.	114	84	107	119	69

[a]Total volume, in cc, of pea aphids collected on 24 September was recorded as there were too many pea aphids to justify counting.

The test shows that samples of alfalfa weevil grubs can be taken at any time of day. The results also show that the area of a field sampled is extremely important and that there is marked field-to-field variation in alfalfa pests. Pooled samples from at least five areas of each field, as suggested by Blickenstaff (*op. cit.*), should be the minimum used to sample a field. Later work has shown that at least five fields should be sampled to derive reliable estimates of tarnished plant bug populations in alfalfa on a single date (Romero 1972). The time of day that the sample was made will also affect the results, except for the grubs, with date or weather. There was no single "best" time for sampling all species. The alfalfa weevil adults, tarnished plant bug, and meadow spittlebug were all most abundant in the early morning and late afternoon. Daylight appears to be an

important factor with these insects. Their numbers dropped between 18:00 and 21:00 hours on 24 September when the temperature dropped from 51° to 48°F, respectively. It appears that samples made on warm evenings give the most reliable sweep net samples of the complex. The only exception to this would be an underestimation of the population of the pea aphid. A striking result of the present test is that a 40-fold error in population estimates from sweep samples is possible if time and area sampled are ignored.

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